Final Report

Study Title

Determination of the Concentration c   I in Dosing Solutions

Author

Study Completion Date

June 24, 2010

Test Facility

Nisso Chemical Analysis Service Co., Ltd. (NCAS)

Odawara Laboratory

345 Takada, Odawara, Kanagawa 250-0216, Japan

Sponsor

Study Number

NCAS 10-022

GLP Compliance Statement

Study Number :   NCAS 10-022

Study Title :   Determination of the Concentration c   iI in Dosing Solutions

This study was carried out in accordance with the following good laboratory practice regulation.

- Standard for the test facility conducting tests concerning new chemical substances, etc. (Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, No. 1121003, November 21, 2003; Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, No. 3, November 17, 2003; Environmental Policy Bureau, Ministry of the Environment, No. 031121004), Final Amendment: July 4, 2008

This study was carried out in accordance with the following procedure and the final report was prepared faithfully and consistently with the raw data obtained.

Study Director :   (signature)_____ (seal) _____(June 24, 2010)_____

Kenji Miya

Odawara Laboratory

Nisso Chemical Analysis Service Co., Ltd.

The original signature page of GLP Compliance Statement is shown in page 3.

Original GLP Compliance Statement

The English translation of GLP Compliance Statement is shown in page 2.

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GLP 適合陳述書

試験番号: NCAS 10-022

試験名: 投与液中の LiFSI 濃度確認試験

この試験は、以下の GLP 規則に従って実施した。

・「新規化学物質等に係る試験を実施する試験施設に関する基準について」(平成 15 年 11 月 21 日 薬食発第
1121003 号、平成 15・11・17 製局第 3 号、環保企発第 031121004 号)、最終改正 平成 20 年 7 月 4 日

この試験はここに述べられた方法により行われ、この最終報告書は試験実施により得られた生データを正確に反
映したものである。

試験責任者: 宮　賢治   ㊞   6, 24, 2010

宮　賢治
株式会社日曹分析センター 小田原事業所

Quality Assurance Statement

Study Number : NCAS 10-022

Study Title :   Determination of the Concentration   ;I in Dosing Solutions

Quality Assurance inspections of the study referred above were conducted according to the appropriate GLP regulations and the standard operating procedures (SOPs) of the Quality Assurance Unit (QAU).   The results of the inspections were reported to the study director and the facility management on the following dates.

Items inspected	Dates (Month/Day/Year)		
	Inspected	Reported to	
		SD	Management
Study plan	3/19/2010	3/19/2010	3/19/2010
Amendment No.1	4/6/2010	4/6/2010	4/6/2010
Experimental operation			
· Receipt of the dosing solutions	4/12, 13/2010	4/13/2010	4/13/2010
· Preparation of the calibration solutions	3/26, 29/2010	3/29/2010	3/29/2010
· Preparation of the test solutions for LC/MS/MS measurement	4/12, 13/2010	4/13/2010	4/13/2010
· LC/MS/MS measurement	4/12, 13/2010	4/13/2010	4/13/2010
· Storage stability of the reference substance (NMR)	5/12/2010	5/12/2010	5/12/2010
Raw Data	5/21 - 27/2010	5/27/2010	5/27/2010
Draft Report	5/21 - 27/2010	5/27/2010	5/27/2010
Final Report	6/24/2010	6/24/2010	6/24/2010

The QAU found that the study was performed according to the study plan and SOPs, the reported methods and procedures were actually used and the results accurately reflect the recorded data.

QAU Manager: (signature)   (seal)   (June 24, 2010)

                 Ken Watabe

                 Nisso Chemical Analysis Service Co., Ltd.

The original signature page of Quality Assurance Statement is shown in page 5.

Original Quality Assurance Statement

The English translation of Quality Assurance Statement is shown in page 4.

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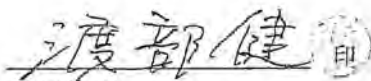
信頼性保証書

試験番号： NCAS 10-022

試 験 名： 投与液中の　　　濃度確認試験

上記試験の信頼性保証の監査または査察を適用 GLP および信頼性保証部門（QAU）の SOP
に基づいて実施した。監査または査察の結果は、以下の日付で試験責任者および運営管理者に
報告した。

監査または査察項目	日付（月/日/年）		
	監査または	報告日	
	査察日	試験責任者	運営管理者
試験計画書	3/19/2010	3/19/2010	3/19/2010
試験計画書変更届 1	4/6/2010	4/6/2010	4/6/2010
実験操作			
・分析試料受領時の処置	4/12, 13/2010	4/13/2010	4/13/2010
・標準溶液の調製	3/26, 29/2010	3/29/2010	3/29/2010
・分析試料の採取および処理	4/12, 13/2010	4/13/2010	4/13/2010
・分析	4/12, 13/2010	4/13/2010	4/13/2010
・安定性確認（NMR）	5/12/2010	5/12/2010	5/12/2010
生データ	5/21-27/2010	5/27/2010	5/27/2010
報告書草案	5/21-27/2010	5/27/2010	5/27/2010
最終報告書	6/24/2010	6/24/2010	6/24/2010

QAU は、この試験が試験計画書および SOP に従って行われ、報告された方法や手段が実際に
使われたものであり、結果は記録されたデータを正確に反映していることを確認した。

QAU 責任者　　渡部 健　　印　　　　　　2010 年 6 月 29 日
　　　　　　　渡部　健
　　　　　（株）日嘗分析センター

Study Information

Study Number :   NCAS 10-022

Study Title :   Determination of the Concentration of   in Dosing Solutions

Report Number : NCAS 10-022

Sponsor :

Test Facility :   Nisso Chemical Analysis Service Co., Ltd.   Odawara Laboratory

345 Takada, Odawara, Kanagawa 250-0216, Japan

TEL   0465-42-8201   FAX   0465-42-3586

Study Director :   Kenji Miya

Experimenter :   Kenji Miya (LC/MS/MS Measurement and Calculation of Concentration)

Shinpei Tsushima (Identity and Storage Stability of the Reference Substance)

Junko Tashiro (Receipt of Dosing Solutions, Preparation of Test Solutions for LC/MS/MS

Measurement and Storage Stability   in Dosing Solutions)

Study Initiation Date :   March 19, 2010

Experimental Start Date :   March 24, 2010

Experimental Completion Date : May 12, 2010

Study Completion Date :   June 24, 2010

Data Requirement :   None

Archiving :   All the documents of this study will be retained for 10 years after the end of this study in the
archives of the test facility.   The management of the documents after that will be determined
by mutual consultation between the Sponsor and Nisso Chemical Analysis Service Co., Ltd. at
that time.   The reference substance will be returned after the end of this study.

Deviation from SOPs and Study Plan :

Trifluoroacetic acid used as a chemical shift standard for NMR measurement was over the
expiry date specified in the SOP.   However, it was judged that it was not influenced to the
results because trifluoroacetic acid was not decomposed from the result of NMR measurement.

Circumstance/matter that Affect the Reliability of the Test Result :

There was no environmental agent which influenced the reliability of the test result.

Table of Contents

Contents of Tables and Figures

Summary

This study was done in order to confirm the concentrations of   in dosing solutions which were used in the repeated dose 28-day oral toxicity study carried out by Safety Research Institute for Chemical Compounds Co., Ltd. (toxicity study No. SR09244).   The concentrations of   in the dosing solutions were measured by LC/MS/MS and the results were as follows.

Sample	Nominal Concentration (mg/mL)	Concentration of 1st Dosing Solution (mg/mL)	Concentration of 2nd Dosing Solution (mg/mL)
3 mg/mL solution	3	3.00	3.03
10 mg/mL solution	10	10.5	10.1
30 mg/mL solution	30	30.3	29.8

Furthermore, the identity and storage stability tests of the reference substance and the storage stability test of   in the dosing solution were conducted in this study.

The   spectrum of the reference substance measured at the start of the experiment was almost the same as the spectrum supplied by the Sponsor.   Thus, it was confirmed that the reference substance was   .   The   spectrum of the reference substance measured at the end of the experiment was the same as the spectrum measured at the start of the experiment.   Thus, it was judged that the reference substance was stable during the experimental period.

The storage stability solutions at concentrations of 1 and 100 mg/mL were prepared.   These solutions were kept in a refrigerator for 10 days and then they were placed at room temperature for 4 hours.   The concentration of   I in each storage stability solution was measured before and after storage, and the remaining rates [(Concentration of   after storage / Concentration of   I before storage)×100] were 102.1% for the 1 mg/mL solution and 102.5% for the 100 mg/mL solution.   Thus, it was judged that   I in dosing solutions was stable for 10 days in the refrigerator and for 4 hours at room temperature because the criterion of the stability (100.0±10.0%) was met.   The storage temperatures were 3.1 to 7.1°C (refrigerator) and 23.1 to 24.3°C (room temperature).

Introduction

This study was done in order to confirm the concentrations of   in dosing solutions which were used in the repeated dose 28-day oral toxicity study carried out by Safety Research Institute for Chemical Compounds Co., Ltd.   Furthermore, the identity and storage stability tests of   and the storage stability test of   in dosing solutions were conducted in this study.

Materials and Methods

1.   Dosing Solutions

The dosing solutions were received on the following schedules.   The dosing solutions were solutions in water purified according to the Japanese Pharmacopoeia.   All dosing solutions were immediately analyzed after receipt.

	Sample	Nominal Concentration (mg/mL)	Received Date
1st receipt	3 mg/mL solution	3	April 12, 2010
	10 mg/mL solution	10	
	30 mg/mL solution	30	
2nd receipt	3 mg/mL solution	3	May 7, 2010
	10 mg/mL solution	10	
	30 mg/mL solution	30	

2. Substance to Measure
    Name :
    Abbreviated name :
    CAS No. :
    Molecular formula :
    Structure :

    Molecular weight :
    Appearance :
    Stability :

3. Reference Substance
    Name :
    Lot No. :
    NCAS retrieval No. :   STD-1150
    Supplier :
    Received date :   January 18, 2010
    Received weight :   420 g
    Purity :
    Storage condition :   Stored in the refrigerator with a polypropylene vessel
    Appearance :   Transparent liquid
    Other information was described in section 2.

4. Reagents and Apparatus
    Distilled water, acetonitrile :   for HPLC, Wako Pure Chemical Industries, Ltd.
    Purified water :   Purified water according to the Japanese Pharmacopoeia, Yakuhan
                             Pharmaceutical Co., Ltd.
    Formic acid :   Guaranteed, Wako Pure Chemical Industries, Ltd.
    Deuterium oxide :   for NMR, ISOTEC
    Trifluoroacetic acid :   for HPLC, Wako Pure Chemical Industries, Ltd.
    Balance :   XP205, Mettler-Toledo International Inc.
                            EB-3200H and EB-3300H, Shimadzu Corp.
    Liquid chromatograph mass spectrometer (LC/MS/MS) :   Acquity/Quattro microAPI, Waters Corp.
    Nuclear magnetic resonance (NMR) apparatus :   JNM-ECA500, JEOL Ltd.

5. Analytical Method for the Determination of the Concentrations of   in the Dosing Solutions

5.1. LC/MS/MS Conditions

Column :	Acquity UPLC BEH C18, 2.1 mm i.d. × 50 mm
	Particle diameter 1.7 μm (Waters Corp.)
Mobile phase :	Acetonitorile + 0.1% Formic acid in water (v/v) = 50 + 50 (v/v)
Flow rate :	0.3 mL/min
Column temperature :	40℃
Autosampler tray temperature :	4℃
Injection volume :	5 μL
Ionization mode :	ESI, Negative
Monitored ion :	180.33 > 96.90 (Quantification), 180.33 > 77.84 (Confirmation)

5.2. Preparation of Calibration Solutions

About 21 mg of the reference substance was accurately weighed into a 50-mL volumetric flask and dissolved in 50% acetonitrile in water to prepare a 100 mg/L (corrected by the purity) standard solution (Solution No. SS-0).   Five milliliter of this solution was taken into a 50-mL volumetric flask and diluted with 50% acetonitrile in water to prepare a 10 mg/L standard solution (Solution No. SS-10). The 10 mg/L standard solution was further diluted with 50% acetonitrile in water to make the calibration solutions listed in the following table. These calibration solutions were prepared at the time used and the unique ID, preparation date, was added to the solution number. The weights of the reference substance and the concentrations of the calibration solutions are shown in Table 2, Table 5 and Table 8.

Solution No.	Volume of 10 mg/L Solution (mL)	Final Volume(mL)	Nominal Concentration (mg/L)
SS-1	2.0	20	1
SS-2	4.0	20	2
SS-3	6.0	20	3
SS-4	8.0	20	4
SS-5	10.0	20	5

5.3. Measurement of the Calibration Solutions and Calculation of the Calibration Equation

The calibration solutions obtained from section 5.2 were injected into the LC/MS/MS described in section 5.1 in order to determine the peak areas of   . The calibration curve was prepared by plotting the peak areas *vs.* the concentrations of the calibration solutions by using Mass Lynx v 4.1 which is the software of the LC/MS/MS, and the calibration equation and the correlation coefficient were calculated with the same software. The accuracy (%) for each calibration solution was calculated by using Microsoft Excel (SP3). The weighting of 1/x was carried out. The calibration equation and the correlation coefficient are shown in Table 2, Table 5 and Table 8.

The criteria for the calibration curve were described below.
  1. The correlation coefficient is more than 0.990.
  2. The accuracy for the lowest concentration solution is within ±20%, and that for other solutions are within ±15%.

5.4. Apparatus Check before Measurement

Before the measurement of the test solutions, the calibration solution of the highest concentration prepared in section 5.2 (SS-5) was measured 3 times by LC/MS/MS and then the peak areas of   were determined. When the coefficient of variation of peak areas was less than 10%, it was judged that the LC/MS/MS was normal.

5.5.   Measurement of Dosing Solutions

Each dosing solution received in section 1 was measured twice.   The dosing solutions were diluted according to the following procedure and the diluted solutions were used as the test solutions for LC/MS/MS measurement.

Dosing solution at 3 mg/mL

One milliliter of the dosing solution was taken into a 25-mL volumetric flask and diluted with 50% acetonitrile in water.   Five milliliter of the diluted solution was diluted with 50% acetonitrile in water to make a 25 mL solution.   Furthermore, 4.0 mL of the solution was diluted with 50% acetonitrile in water to make a 25 mL solution.

Dosing solution at 10 mg/mL

Two milliliter of the dosing solution was taken into a 20-mL volumetric flask and diluted with 50% acetonitrile in water.   One milliliter of the diluted solution was diluted with 50% acetonitrile in water to make a 50 mL solution.   Furthermore, 4.0 mL of the solution was diluted with 50% acetonitrile in water to make a 25 mL solution.

Dosing solution at 30 mg/mL

One milliliter of the dosing solution was taken into a 25-mL volumetric flask and diluted with 50% acetonitrile in water.   One milliliter of the diluted solution was diluted with 50% acetonitrile in water to make a 50 mL solution.   Furthermore, 4.0 mL of the solution was diluted with 50% acetonitrile in water to make a 25 mL solution.

The test solutions prepared above were measured on the LC/MS/MS conditions described in section 5.1 and the peak areas of   were determined.   The concentrations of   in the test solutions were calculated by Mass Lynx v 4.1 which is the software of the LC/MS/MS and then the concentrations of   in the dosing solutions were calculated by the following equation.   The average concentration was regarded as the concentration of the dosing solution and its significant figure of three figures was adopted. When the coefficient of variation of the duplicate analyses was less than 10%, it was judged that the measurement result was effective.

$$\text{Concentration of} \quad (\text{mg/mL}) = \frac{\text{Concentration of the test solution (mg/L)} \times \text{Dilution factor}}{1000}$$

The blank solution, 1.0 mL of the purified water diluted like the dosing solution at 3 mg/mL, was measured on the LC/MS/MS conditions described in section 5.1 to confirm that no interfering peak is observed at the retention time of the test substance (n=1).

6.   Identity and Storage Stability of the Reference Substance

6.1.   Identity of the Reference Substance

An aliquot of the reference substance (101.54 mg) was weighed and 0.6 mL of deuterium oxide and 10 µL of trifluoroacetic acid were added to the reference substance.   The   spectrum of this solution was measured on the following conditions in the experimental start date.   This spectrum was compared with the NMR spectrum supplied by the Sponsor to confirm the identity.

<Conditions>

Nuclear :

Pulse sequence :   Single pulse

Temperature :   25℃

Scan :   8

Chemical shift standard :   Trifluoroacetic acid (chemical shift -76.5 ppm)

6.2.   Storage Stability of the Reference Substance

Deuterium oxide and trifluoroacetic acid were added to the reference substance (104.78 mg) like section 6.1 and the   spectrum was measured in the experimental completion date (the same NMR conditions as section 6.1).   The spectrum was compared with the NMR spectrum measured in the experimental start date to confirm the storage stability.   When both of chemical shifts were in agreement, it was judged that the reference substance was stable during the experimental period.

7.   Storage Stability of   I in the Dosing Solutions

The storage stability of   in the dosing solutions was confirmed in this study.   The storage stability solutions at concentrations of 1 and 100 mg/mL were prepared.   These solutions were kept in the refrigerator for 10 days and then they were placed at room temperature for 4 hours.   The concentrations of   in the storage stability solutions were measured before and after storage and the remaining rate was calculated.

7.1.   Preparation of the Storage Stability Solution

About 4.17 g of the reference substance was weighed precisely and dissolved in the purified water to make a 10 mL solution (100 mg/mL).   This solution was further diluted with the purified water to prepare a 1 mg/mL solution.

7.2.   Measurement of the Storage Stability Solution

Each storage stability solution was diluted according to the following procedure and the diluted solution was measured on the LC/MS/MS conditions described in section 5.1 (n = 2).

Storage stability solution at 1 mg/mL

One milliliter of the storage stability solution was taken into a 50-mL volumetric flask and diluted with 50% acetonitrile in water.   Four milliliter of this solution was diluted with 50% acetonitrile in water to make a 25 mL solution.

Storage stability solution at 100 mg/mL

One milliliter of the storage stability solution was taken into a 100-mL volumetric flask and diluted with 50% acetonitrile in water.   One milliliter of this solution was diluted with 50% acetonitrile in water to make a 50 mL solution.   Furthermore, 4.0 mL of the solution was diluted with 50% acetonitrile in water to make a 25 mL solution.

The storage stability solutions were kept in a refrigerator for 10 days and then they were placed at room temperature for 4 hours.   The concentrations of   I in the storage stability solutions were measured before and after storage, and the remaining rates were calculated according to section 7.3.

7.3.   Criteria of Storage Stability

The remaining rate was calculated in the following equation.   When the remaining rate was 100.0± 10.0%, it was judged that   in the dosing solution was stable.

$$\text{Remaining rate (\%)} = \frac{\text{Average concentration after storage (10 days in refrig.+4 hours at r.t.)}}{\text{Average concentration before storage}} \times 100$$

refrig. : refrigerator, r.t. : room temperature

8.   Application Software and Rounding of Numbers

The calibration equation and the coefficient of correlation were calculated by Mass Lynx v 4.1 (LC/MS/MS software) and Microsoft Excel (SP3) was used for other calculations.

The significant figure was adopted four figures for the concentration of the test solution and three figures for the concentration of the dosing solution.   The standard deviation was shown to the same figure as the average concentration.   The coefficient of variation and the remaining rate were calculated to one decimal place.

Results, Consideration and Conclusion

1. Concentrations of   in the Dosing Solutions

  1.1 First Dosing Solutions

    The result of the apparatus check before measurement is shown in Table 1. The calibration solution at the highest concentration (SS-5) was measured 3 times by LC/MS/MS and the coefficient of variation of the peak areas was 2.4%. Thus, it was judged that the LC/MS/MS was normal.

    The result of the measurement for the calibration solutions is shown in Table 2. The calibration curve is shown in Figure 1 and typical chromatograms for the calibration solutions are shown in Figure 2 and Figure 3. The correlation coefficient of the calibration curve was 0.9947 and the accuracy of all calibration solutions was −7.82 to 8.47%. Thus, these values met the criteria for the calibration curve.

    The result of the measurement for the first dosing solutions is shown in Table 3. Typical chromatograms of the blank and dosing solutions are shown in Figure 4 to Figure 7. Since the coefficients of variation of the concentration for the dosing solutions were 0.7 to 1.9%, the measurement result was effective. The average concentrations are shown below.

Sample	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)
Blank solution	—	No interfering peak was detected.
3 mg/mL solution	3	3.00
10 mg/mL solution	10	10.5
30 mg/mL solution	30	30.3

  1.2 Second Dosing Solutions

    The result of the apparatus check before measurement is shown in Table 4. The calibration solution at the highest concentration (SS-5) was measured 3 times by LC/MS/MS and the coefficient of variation of the peak areas was 1.4%. Thus, it was judged that the LC/MS/MS was normal.

    The result of the measurement for the calibration solutions is shown in Table 5. The correlation coefficient of the calibration curve was 0.9989 and the accuracy of all calibration solutions was −3.68 to 2.92%. Thus, these values met the criteria for the calibration curve.

    The result of the measurement for the second dosing solutions is shown in Table 6. Since the coefficients of variation of the concentration for the dosing solutions were 0.0 to 1.7%, the measurement result was effective. The average concentrations are shown below.

Sample	Nominal Concentration (mg/mL)	Measured Concentration (mg/mL)
Blank solution	—	No interfering peak was detected.
3 mg/mL solution	3	3.03
10 mg/mL solution	10	10.1
30 mg/mL solution	30	29.8

2. Identity and Storage Stability of the Reference Substance

    The   R spectrum of   supplied by the Sponsor is shown in Figure 8 and the spectra of the reference substance measured at the start and end of the experiments are shown in Figure 9 and Figure 10, respectively.

    According to the NMR spectrum supplied by the Sponsor, the chemical shift of   was about 53 ppm when   l was used as the chemical shift standard. In this study, trifluoroacetic acid was used as the chemical shift standard and the chemical shift of   was about 51 ppm. It was judged that the difference in the chemical shifts of   was attributed to the chemical shift standard used. Thus, it was judged that the reference substance was

    The   spectra measured in the experimental start and completion dates were compared mutually and both of the chemical shifts were the same. Thus, it was judged that   was stable during the experimental period.

3. Storage Stability of   in the Dosing Solution

The result of the apparatus check before measurement is shown in Table 7. The calibration solution at the highest concentration (SS-5) was measured 3 times by LC/MS/MS and the coefficients of variation of the peak areas were 0.8% (at the analysis of the storage stability solutions before storage) and 7.8% (at the analysis of the storage stability solutions after storage). Thus, it was judged that the LC/MS/MS was normal.

The result of the measurement for the calibration solutions is shown in Table 8. At the analysis of the storage stability solutions before storage, the correlation coefficient of the calibration curve was 0.9994 and the accuracy of all calibration solutions was -3.16 to 4.12%. At the analysis of the storage stability solutions after storage, the correlation coefficient of the calibration curve was 0.9982 and the accuracy of all calibration solutions was -4.26 to 4.17%. Thus, these values met the criteria for the calibration curve.

The result of the measurement for the storage stability of   in the dosing solution is shown in Table 9. Typical chromatograms of the storage stability solution before storage are shown in Figure 11 and Figure 12. Typical chromatograms of the storage stability solution after storage are shown in Figure 13 and Figure 14. Since the coefficients of variation of the concentration for the storage stability solutions were 0.6 to 3.9%, the measurement result was effective.

The remaining rates of the storage stability solutions at concentrations of 1 and 100 mg/mL were 102.1% and 102.5%, respectively. Since these values met the criterion of the storage stability (100.0± 10.0%), it was judged that   in the dosing solutions was stable for 10 days in the refrigerator and for 4 hours at room temperature. The storage temperatures were 3.1 to 7.1°C in the refrigerator and 23.1 to 24.3°C at room temperature.

Table 1　Result of the apparatus check before measurement : The 1st dosing solutions (Measurement : April 12, 2010)

Sample ID	Peak Area	Average	Standard Deviation	Coefficient of Variation (%)
100412-SS-5	8737	8878	215	2.4
	8771			
	9126			

Table 2　Result of the measurement for the calibration solutions : The 1st dosing solutions (Measurement : April 12, 2010)

Sample ID	Concentration (mg/L)	Peak Area	Calculated Concentration (mg/L)	Accuracy (%)
100412-SS-1	1.010	2102.8	0.9314	-7.82
100412-SS-2	2.021	3833.5	2.131	5.45
100412-SS-3	3.031	5501.6	3.288	8.47
100412-SS-4	4.042	6464.2	3.955	-2.14
100412-SS-5	5.052	7756.9	4.851	-3.98

Weight of the Reference Substance :　　21.05 mg

Calibration Equation :　　　　　　　　$y = 1442.51 x + 759.24$ (weighting : 1/x)

Correlation Coefficient :　　　　　　　0.9947

Table 3　Result of the measurement for the dosing solutions : The 1st dosing solutions (Measurement : April 12, 2010)

Sample ID	Nominal Concentration (mg/mL)	Dilution Factor	Peak Area	Calculated Concentration (mg/L)	Concentration in Dosing Solution			
					Individual (mg/mL)	Average (mg/mL)	Standard Deviation (mg/mL)	Coefficient of Variation (%)
3mg/ml-1-1-2	3	781.3	6270.9	3.821	2.99	3.00	0.02	0.7
3mg/ml-1-2-2			6323.2	3.857	3.01			
10mg/ml-1-1-2	10	3125	5702.3	3.427	10.7	10.5	0.2	1.9
10mg/ml-1-2-2			5545.9	3.318	10.4			
30mg/ml-1-1-2	30	7813	6299.7	3.841	30.0	30.3	0.5	1.7
30mg/ml-1-2-2			6423.5	3.927	30.7			

Table 4 Result of the apparatus check before measurement : The 2nd dosing solutions (Measurement : May 7, 2010)

Sample ID	Peak Area	Average	Standard Deviation	Coefficient of Variation (%)
100507-SS-5	7396	7514	108	1.4
	7536			
	7609			

Table 5 Result of the measurement for the calibration solutions : The 2nd dosing solutions (Measurement : May 7, 2010)

Sample ID	Concentration (mg/L)	Peak Area	Calculated Concentration (mg/L)	Accuracy (%)
100507-SS-1	1.003	1959.7	0.9663	-3.68
100507-SS-2	2.006	3543.3	2.065	2.92
100507-SS-3	3.010	5013.9	3.085	2.51
100507-SS-4	4.013	6405.1	4.050	0.93
100507-SS-5	5.016	7606.3	4.883	-2.65

Weight of the Reference Substance : 20.90 mg
Calibration Equation : $y = 1441.73 x + 566.632$ (weighting : 1/x)
Correlation Coefficient : 0.9989

Table 6 Result of the measurement for the dosing solutions : The 2nd dosing solutions (Measurement : May 7, 2010)

Sample ID	Nominal Concentration (mg/mL)	Dilution Factor	Peak Area	Calculated Concentration (mg/L)	Concentration of in Dosing Solution			
					Individual (mg/mL)	Average (mg/mL)	Standard Deviation (mg/mL)	Coefficient of Variation (%)
3mg/ml-2-1-2	3	781.3	6229.6	3.928	3.07	3.03	0.05	1.7
3mg/ml-2-2-2			6095.0	3.834	3.00			
10mg/ml-2-1-2	10	3125	5264.3	3.258	10.2	10.1	0.1	1.0
10mg/ml-2-2-2			5215.4	3.224	10.1			
30mg/ml-2-1-2	30	7813	6069.0	3.816	29.8	29.8	0.0	0.0
30mg/ml-2-2-2			6065.5	3.814	29.8			

Table 7 Result of the apparatus check before measurement : Storage stability of I in the dosing solution (Measurement : March 26 and April 5, 2010)

	Measurement : March 26, 2010				Measurement : April 5, 2010				
Sample ID	Peak Area	Average	Standard Deviation	Coefficient of Variation (%)	Sample ID	Peak Area	Average	Standard Deviation	Coefficient of Variation (%)
100326-SS-5	8261	8194	64	0.8	100405-SS-5	7379	8054	628	7.8
	8188					8160			
	8134					8622			

Table 8 Result of the measurement for the calibration solutions : Storage stability of in the dosing solution (Measurement : March 26 and April 5, 2010)

	Measurement : March 26, 2010				Measurement : April 5, 2010				
Sample ID	Concentration (mg/L)	Peak Area	Calculated Concentration (mg/L)	Accuracy (%)	Sample ID	Concentration (mg/L)	Peak Area	Calculated Concentration (mg/L)	Accuracy (%)
100326-SS-1	1.011	2219.9	0.9794	-3.16	100405-SS-1	1.016	2516.4	0.9724	-4.26
100326-SS-2	2.023	3846.2	2.106	4.12	100405-SS-2	2.031	4560.7	2.116	4.17
100326-SS-3	3.034	5208.5	3.051	0.56	100405-SS-3	3.047	6279.5	3.078	1.02
100326-SS-4	4.045	6586.8	4.006	-0.97	100405-SS-4	4.063	8224.0	4.166	2.54
100326-SS-5	5.057	8061.6	5.028	-0.57	100405-SS-5	5.078	9539.1	4.902	-3.47

Weight of Reference Substance : 21.07 mg
Calibration Equation : $y = 1442.96 x + 806.657$ (weighting : 1/x)
Correlation Coefficient : 0.9994

Weight of Reference Substance : 21.16 mg
Calibration Equation : $y = 1787.1 x + 778.666$ (weighting : 1/x)
Correlation Coefficient : 0.9982

Table 9 Result of the measurement for the storage stability solutions : Storage stability of I in the dosing solution (Measurement : March 26 and April 5, 2010)

Nominal Concentration (mg/mL)	Storage Condition	Analysis Date	Dilution Factor	Peak Area	Calculated Concentration (mg/L)	Concentration of in the Storage Stability Solution				Remaining Rate (%)
						Individual (mg/mL)	Average (mg/mL)	Standard Deviation (mg/mL)	Coefficient of Variation (%)	
1	10 days in refrigerator + 4 hours at room temperature	April 5, 2010	312.5	6607.6	3.262	1.019	0.994	0.036	3.6	102.1
				6316.7	3.099	0.9684				
	Before storage	March 26, 2010		5179.2	3.030	0.9469	0.974	0.038	3.9	
				5430.1	3.204	1.001				
100	10 days in refrigerator + 4 hours at room temperature	April 5, 2010	31250	6243.4	3.058	95.56	98.1	3.6	3.7	102.5
				6536.0	3.222	100.7				
	Before storage	March 26, 2010		5206.6	3.049	95.28	95.7	0.6	0.6	
				5247.3	3.077	96.16				

Compound name:
Correlation coefficient: r = 0.994705, r^2 = 0.989439
Calibration curve: 1442.51 * x + 759.24
Response type: External Std, Area
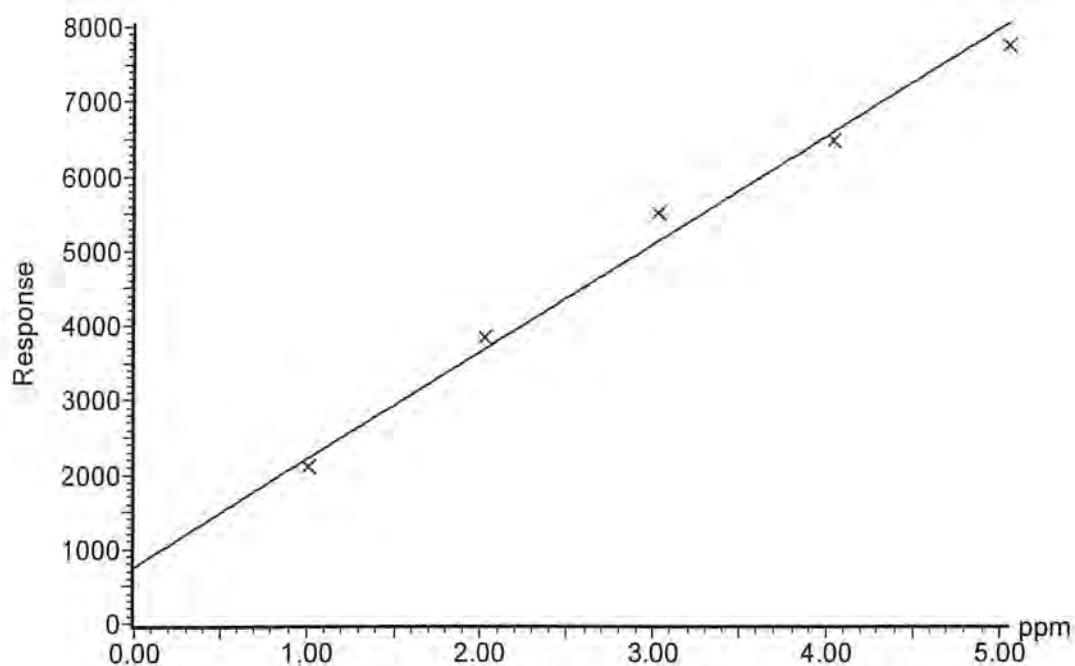Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 1   Typical calibration curve (Measurement : April 12, 2010)

1000412-004
STD 1 ppm 100412-SS-1

Figure 2   Typical chromatogram of the calibration solution (1.010 mg/L, Measurement : April 12, 2010)

Figure 3   Typical chromatogram of the calibration solution   (5.052 mg/L, Measurement : April 12, 2010)

Figure 4   Typical chromatogram of the blank solution (Measurement : April 12, 2010)

Figure 5   Typical chromatogram of the dosing solution at 3 mg/mL (Measurement ; April 12, 2010)

Figure 6   Typical chromatogram of the dosing solution at 10 mg/mL (Measurement : April 12, 2010)

Figure 7 Typical chromatogram of the dosing solution at 30 mg/mL (Measurement : April 12, 2010)

Figure 8   spectrum of   supplied by the Sponsor

Figure 9   spectrum of the reference substance in the experimental start date

Figure 10   R spectrum of the reference substance in the experimental completion date

Figure 11   Typical chromatogram of the storage stability solution at 1 mg/mL
(before storage, Measurement : March 26, 2010)

Figure 12   Typical chromatogram of the storage stability solution at 100 mg/mL
(before storage, Measurement : March 26, 2010)

Figure 13   Typical chromatogram of the storage stability solution at 1 mg/mL
(after storage, Measurement : April 5, 2010)

Figure 14   Typical chromatogram of the storage stability solution at 100 mg/mL
(after storage, Measurement : April 5, 2010)

Authenticity of Translation

I declare that the original Japanese final report (Report No. NCAS 10-022) is translated into English consistently.

Translated by : _Kenji Miya_   _April 27, 2012_

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